
Dynamics of CRISPR-Cas9 genome interrogation in living cells.

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Public Summary:

The RNA-guided CRISPR-associated protein Cas9 is used for genome editing, transcriptional modulation, and live-cell imaging. Cas9-guide RNA complexes recognize and cleave double-stranded DNA sequences on the basis of 20-nucleotide RNA-DNA complementarity, but the mechanism of target searching in mammalian cells is unknown. Here, we use single-particle tracking to visualize diffusion and chromatin binding of Cas9 in living cells. We show that three-dimensional diffusion dominates Cas9 searching in vivo, and off-target binding events are, on average, short-lived (<1 second). Searching is dependent on the local chromatin environment, with less sampling and slower movement within heterochromatin. These results reveal how the bacterial Cas9 protein interrogates mammalian genomes and navigates eukaryotic chromatin structure.

Scientific Abstract:

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